

WO 2005/045014

PCT/AU2004/001549

- 10 -

CLAIMS

1. A method of producing large quantities of baculovirus including  
inoculating caterpillar larvae with a baculovirus inoculum;  
5 incubating inoculated caterpillar larvae;  
harvesting baculovirus occlusion bodies from the infected caterpillar larvae;  
extracting occlusion derived virus from the occlusion bodies;  
inoculating a culture of host insect cells with an inoculum of occlusion derived  
virus;  
10 incubating virus/cell culture; and  
harvesting baculovirus from the incubated virus/cell culture.
2. A method of producing large quantities of baculovirus as claimed in claim 1  
wherein the incubation of the virus/cell culture is for a period of time that enables four  
15 or five passages of Baculovirus.
3. A method of producing large quantities of Baculovirus as claimed in claim 1 or  
2, wherein the Baculovirus is selected from any one of the following group:  
*Helicoverpa armigera* SNPV, *Helicoverpa zea* SNPV, *Spodoptera frugiperda* MNPV,  
20 *Anticarsia gemmatilis* MNPV, *Autographa californica* MNPV, *Anagrapha falcifera*  
MNPV, *Lymantria dispar* MNPV, *Bombyx mori* MNPV, *Spodoptera exigua* MNPV,  
*Trichoplusia ni* MNPV, *Orgyia pseudotsugata* MNPV and *Buzura suppressaria*  
SNPV.

WO 2005/045014

PCT/AU2004/001549

- 11 -

4. A method of producing large quantities of Baculovirus as claimed in any one of claims 1 to 3, wherein the baculovirus is a *Helicoverpa armigera* isolate.

5. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein there is more than one step of producing baculovirus from larvae in order to produce a suitable amount of occlusion bodies working stock.

6. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein the baculovirus are produced from larvae in an initial step to form an occlusion bodies master stock, the occlusion bodies master stock is then used to provide inoculum for the production of occlusion bodies working stocks.

7. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein an occlusion bodies working stock has approximately  $2 \times 10^{12}$  occlusion bodies whereas occlusion bodies master stock has approximately  $10^9$  occlusion bodies.

8. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein the occlusion derived virus (ODV) is inoculated in the cell culture at a relatively high MOI.

9. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein an inoculum of occlusion derived virus is

WO 2005/045014

PCT/AU2004/001549

- 12 -

obtained from as low as  $2.5 \times 10^{10}$  occlusion bodies and introduced into a ten litre bioreactor containing  $5 \times 10^5$  cells per ml, the culture is then progressively scaled up from a 10 litre volume (P1) to a 100 litre volume (P2), then to a 1,000 litre volume (P3) and finally a 10,000 litre volume (P4); wherein the 10 litre culture produces  
5 approximately  $10^7$  PFU (Baculovirus) per ml, the 10,000 litre culture has an approximate cell density between  $1.5\text{--}2.0 \times 10^9$  cells per litre and a  $2.5 \times 10^{11}$  OB per litre (which is approximately 150 OB per cell) and the OB has a LC50 against heliothis caterpillars of between 0.2-1.0 OB per  $\text{mm}^2$ .

10 10. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein the occlusion derived virus is extracted using alkali to lyse the occlusion bodies and the resultant viral particles are stabilized in an appropriate buffering media.

15 11. A method of producing large quantities of Baculovirus as claimed in any one of the abovementioned claims, wherein the method of extraction includes mixing an alkaline solution with an OB suspension and incubating the mixture for a period of time and at a temperature that separates the viral particles, the ODV are then suspended in a stabilizing media; the ODV is extracted without the use of a trypsin  
20 treatment and without the use of serum in the VPM3 media.

12. A Baculovirus product produced from the method as claimed in any one of the preceding claims.